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Genetic basis for the adaptation of Anolis lizards to different thermal microhabitats

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論文題目	Genetic basis for the adaptation of *Anolis* lizards to different thermal microhabitats (アノールトカゲにおける異なる温度環境への適応の遺伝的基盤の解明)
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論文内容の要旨

Introduction

Ecological niche partitioning that allows phenotypic segregation is one of the most important factors promoting ecological specialization, and results in the coexistence of locally adapted phenotypes (Ravigne *et al*. 2009). Species habitats are composed of multidimensional niche axes, including temperature (Hutchinson 1978). Although temperature is a primary factor limiting performances and survival of organisms, particularly ectotherms (Stevenson 1985), some ectotherms coexist by partitioning thermal habitats using temperature as their niche axis (Ruibal 1961; Cádiz *et al.* 2013; Hertz *et al*. 2013). While the traits associated with thermal biology, such as thermal tolerances, are phylogenetically conserved across lineages (Kellermann *et al*. 2012; Araújo *et al*. 2013; Hoffmann *et al*. 2013; Buckley *et al*. 2015). The mechanisms that explain how these species are able to inhabit distinct thermal microhabitats are not well understood. In addition to an important habitat axis, temperature is one of the major determinants for the geographic range of species (Bozinovic *et al*. 2011) as well as the fates of species in response to recent global warming (Hoffmann & Sgrò 2011). Therefore, revealing the genetic basis underlying mechanisms of adaptation to temperature environments is important not only to understand evolutionary adaptation of ectotherms to thermal environments but also how biodiversity has been or will be maintained locally and globally through time.

Anolis lizards have diversified in the Greater Antilles through the divergence and convergence of arboreal habitat specialists, or ecomorph, across the islands (Williams 1972; Losos 2009). Nevertheless, the concept of ecomorphs alone, which have lead to the production of six types of habitat specialists across four islands, can explain *Anolis* diversity only partially. Several studies indicated the thermal properties of *Anolis* lizards to be evolutionarily labile (Hertz *et al*., 2013; Cádiz *et al*. submitted), the emergence of thermal habitat specialists might be another important mechanism contributing to *Anolis* diversification. In fact, phylogenetically closely related species in Cuba, *Anolis allogus*, *A. homolechis*, and *A. sagrei*, select the distinct thermal microhabitats among the species, and coexist in the same community throughout Cuban island (Ruibal, 1961; Cádiz *et al*. 2013). *A. allogus* inhabit shaded forests, where direct sunlight barely reaches the forest floor. *A. homolechis* live along the edge of the forests, and *A. sagrei* inhabit open forests or human-made architectures. The latter two species have the access to the sunlight, and their habitats become hotter than the habitat of *A. allogus*. Because of the distinct characteristics in their habitats and behaviors, *A. allogus*, *A. homolechis*, and *A. sagrei* achieve different average body temperatures in the wild, having relatively low, intermediate, and high body temperatures, respectively (Ruibal 1961). In contrast to their thermal ecology, how these species have adapted to different thermal microhabitats and, particularly, its underlying genetic basis are not well understood.

In order to understand the genetic basis of adaptation to different thermal habitats, I studied these *Anolis* lizards based on the two perspectives: behavioral thermoregulations (**chapter I**), and regulations by gene expressions changes (**chapter II**). In this thesis, I have comprehensively studied the genetic basis of thermal adaptation, and demonstrated the divergence in the genetic basis associated with thermal adaptation in the three *Anolis* lizards.

Chapter I

Among thermoregulatory behaviors, heat avoidance is important for ectotherms to avoid overheating particularly in tropics, where the ambient temperatures stay warm through out the year (Price-Rees *et al.* 2013). The activation of a molecular heat sensor, transient receptor potential ion channel ankyrin 1 (TRPA1), is suggested to elicit heat avoidance behaviors (Rosenzweig *et al.* 2008; Kohno *et al.* 2010; Saito *et al.* 2012). These studies have been conducted only on phylogenetically distant taxa, and how the divergence in the function of TRPA1 and heat avoidance behaviors influences species' thermal habitat selection in phylogenetially closely related species has been unexplored. In chapter I, I have investigated whether behavioral and molecular heat sensitivities have diverged among the three *Anolis* lizards, which inhabit distinct thermal microhabitats. I have conducted the behavioral experiments to measure the temperatures at which the lizards voluntarily escaped from the heated hotplate. The result showed that the temperatures eliciting the heat avoidance behavior were significantly lower in *A. allogus* than *A. homolechis* and *A. sagrei* (Fig. 1a). Furthermore, after confirming the divergence

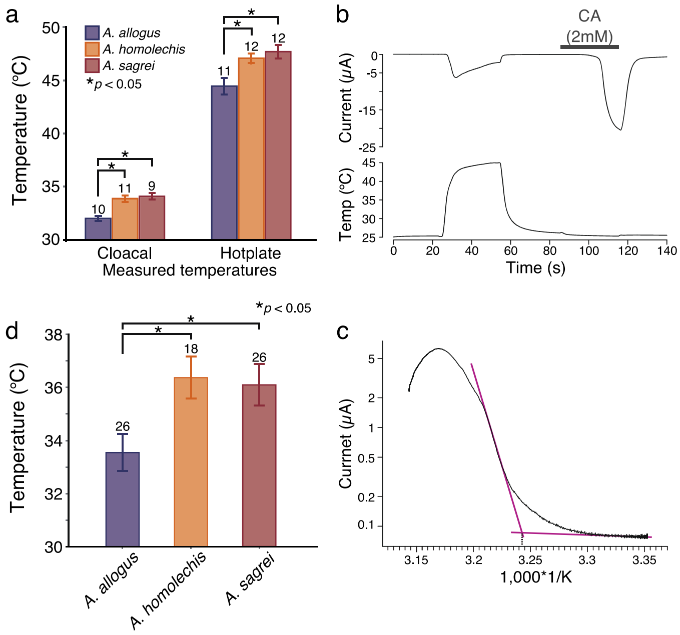

Fig. 1 The behavioral and molecular heat sensitivities of *Anolis* TRPA1. **a**, a comparison of behavioral heat sensitivities, representing the temperatures measured at cloaca of the lizard and at the surface of hotplate. **b**, a representative current and temperature traces for heat and an agonist, CA, stimuli in *A. allogus* TRPA1. **c**, an Arrhanius plot shows thermal threshold of *A. allogus* TRPA1 for the heat stimulus shown in (b). **d**, the mean thermal activation threshold of *Anolis* TRPA1 as estimated by the Arrhenius breakpoints. Sample sizes are indicated on each bar.

in TRPA1 amino acid sequences, I heterologously expressed TRPA1 of each *Anolis* species in *Xenopus laevis* oocytes. Then, I recorded ionic currents using two-electrode voltage clamp method to examine the heat sensitivities of *Anolis* TRPA1. For each species, I determined the average temperature threshold for TRPA1 activation by the first heat stimulation, as calculated with Arrhenius plots (Fig. 1b,c). The result showed that the activation temperatures of TRPA1 were significantly lower in *A. allogus* than *A. homolechis* and *A. sagrei* (Fig. 1d). These results showed the trend, i.e. *A. allogus* as the most heat sensitive species among the three species, as it explains the relationships of thermal microhabitat selections in the wild. This is the first study to show the correlation between behavioral and molecular heat sensitivities in phylogenetically closely related species from distinct thermal habitats.

Chapter II

The genetic basis of physiological mechanisms associated with thermal adaptation has been extensively studied with the advent of next-generation sequencing (NGS) technologies (Porcelli *et al.* 2015). The NGS-based studies, particularly transcriptomics or RNA-seq, give insights into the genetic basis of physiological mechanisms from the perspectives of regulatory changes. In chapter II, I have analyzed the gene expression changes in the three *Anolis* lizards to detect genes associated with adaptation to different thermal microhabitats. Prior to perform RNA-seq, I have kept each species under the constant temperatures of 26 °C and 33 °C for five days in the incubators under 12 hours of light and dark cycles. The temperatures of 26 °C and 33 °C were set as they reflect the average body temperatures of *A. allogus* and *A. sagrei*, respectively, in the wild. Poly-A tailed messenger RNA, extracted from the brain, liver, and skin tissues of each species, were analyzed by RNA-seq on the Illumina Hiseq 2000 platform. Then, I analyzed the change in the gene expression levels in comparisons between the samples from 26 °C and 33 °C treatments for each tissue and species. The results showed that the global gene expression patterns were affected by the two temperature treatments (Fig. 2b), and I identified 397, 813, and 774 differentially expressed genes (DEGs), which are the genes whose expression levels were significantly changed, in *A. allogus*, *A. homolechis*, and *A. sagrei*,

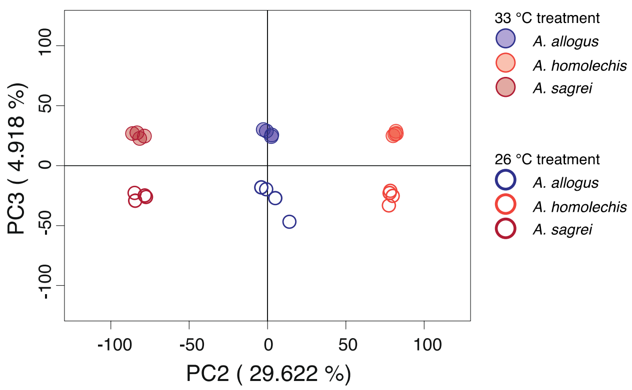

Fig. 2 The principal component analysis for gene expression levels in the brain tissues of the three *Anolis* species. The global gene expression patterns are affected by the incubation treatments at two temperature conditions.

respectively. Gene ontology (GO) analysis showed that the GO term of translation was enriched in *A. homolechis*, as the large numbers of ribosomal protein genes in the DEGs unique to *A. homolechis* brain were upregulated at 33 °C. The upregulation of ribosomal protein genes under heat stress is suggested as the evidence of upregulating protein synthesis to compensate the damaged proteins and to resist the stress (Quinn *et al.* 2011). Thus, *A. homolechis* may be capable of sustaining functional proteins even under the prolonged exposure to the temperature. The result for *A. sagrei* also implied that *A. sagrei* is capable of sustaining functional proteins but less in degree than *A. homolechis*. On the other hand, *A. allogus* did not show any transcriptional responses that are the indicative of such capability. Furthermore, GO analysis showed that a GO term associated with circadian regulation was enriched by DEGs detected in the brain tissues of all three species. Among the genes in the GO term, a gene, *Nr1d1*, showed the opposite expression patterns between *A. allogus*, a thermoconformer species, and *A. sagrei*, a thermoregulator species. Because the habitat temperature fluctuates more widely in the open habitats than in shaded or closed forests throughout the day, the circadian thermoregulation could also be important for adaptation to distinct thermal habitats. The transcriptomic responses in each species provided several evidences of thermal adaptation in each species. While lizards are considered to be vulnerable to climate warming (Huey *et al.* 2010), the study using NGS to understand the genetic basis of thermal adaptation in terrestrial reptiles has never been conducted as far as reported in the review of Porcelli *et al.* (2015). Thus, this is the first study that provided the information on the genetic basis associated with adaptation to different thermal habitats in the terrestrial reptiles.

Discussion

These findings have demonstrated the genetic divergences associated with thermal adaptation in the three Anolis lizards. Both temperatures eliciting heat avoidance behavior and activating TRPA1 were significantly lower in *A. allogus* than in *A. homolechis* and *A. sagrei*. This correlation between behavioral and molecular heat sensitivities in these species suggests that *A. allogus* is genetically constrained to its native or shaded cool forest habitats as opposed to *A. homolechis* and *A. sagrei* that inhabit hotter habitats. In addition, the results from RNA-seq suggest the thermoregulator species, *A. homolechis* and *A. sagrei*, have higher capacity of physiological functions under hotter conditions than the thermoconformer species, *A. allogus*. Therefore, the studies in this thesis provided several evidences of genetic basis important for the adaptation to different thermal habitats.

Hertz *et al.* (2013) indicated thermal biology of *Anolis* species to be evolutionarily labile as various species maintain different body temperatures. While thermal biology is evolutionarily conserved across lineages (Kellermann *et al*. 2012; Araújo *et al*. 2013; Hoffmann *et al*. 2013; Buckley *et al*. 2015), the evolutionarily labile thermal biology is one of the unique characteristics in *Anolis* species. While thermal biology has been extensively studied in *Anolis* lizards (Losos 2009), it has been approached from the studies in the wild or on behavioral experiments, and to what extent the lability is influenced by their genetic background has not been well understood. In this thesis, only three *Anolis* species were studied among the vast diversity in *Anolis* genera, but this is the first study that comprehensively approached the genetic basis of thermal adaptation in *Anolis* lizards. Cádiz *et al*. (submitted) reported that the niche shifting from the closed forest to open habitats has occurred multiple times across Cuban *Anolis* lineages, including the case of the three *Anolis* species studied in this thesis. The studies on other *Anolis* species will provide important evidences to understand the generality of the genetic basis revealed in this thesis, and also to explain the importance of thermal adaptation as a mechanism of *Anolis* diversification. The genetic basis of thermal adaptation unraveled in this thesis will be a foundation stone to understand how biodiversity has been formed, maintained, and respond to the recent climate changes.

論文審査結果の要旨

赤司寛司氏提出の博士論文では､キューバに生息し、異なる温度生息環境で共存している 3 種のアノールトカゲを用いて、温度適応に関わる遺伝的違いを明らかにした。1 章では、温度感知遺伝子である TRPA1 の活性化温度と高温での忌避温度との関係を調べた。森林内部の低温環境に生息する *A. allogus* は、開放環境や森林林縁部の高温環境に生息する *A. homolechis* と *A sagrei* に比べて高温忌避温度は有意に高かった。また、そのときの、TRPA 1 の活性化温度も同様に、*A. allogus* 有意に低かった。この結果は、野外での高温忌避行動は、TRPA1 の感知によって引き起こされていることを示唆している。また、これら 3 種が、26 度と 33 度でどのような遺伝子が発現変動するかを調べるために、RNA-seq を行った。RNA-seq の結果、*A. allogus* で 387, *A. homolechis* で 813, *A sagrei* では 774 の発現変動遺伝子が検出された。GO 解析の結果、*A. homolechis* の脳において translation, 3 種の脳での発現変動遺伝子では circadian regulation of genes が有意に選択された。このことは、*A. homolechis* では、高温にさらされた後、タンパク質修復のためにリボゾームタンパクを高度する RNA が多く発現していることが示唆された。また概日リズムに関する発現変動遺伝子として核受容体遺伝子である Nr1d1 が検出され、*A. allogus* と *A sagrei* で発現のパターンが異なった。この遺伝子は、日周活動時間によって変動する温度耐性に関係があると考えられ、*A. allogus* と *A sagrei* の活動時間の違いと関係があると示唆された。赤司寛司氏の研究では、近縁種で異なる温度環境に適応することで共存しているアノールトカゲを用いることで、温度適応に関わる遺伝子群をはじめて検出した研究となった。この研究は、今後温暖化に伴う生物の進化的反応を探る上で非常に重要な結果を提供している。これらの研究は、赤司寛司氏が主導で行った研究で、赤司寛司氏は研究活動を行うに必要な高度の研究能力と学識を有することを示している。したがって，赤司寛司氏提出の論文は，博士（生命科学）の博士論文として合格と認める。